Research Article

Clinical Implications of EMT in HNSCC: A Review of the Factors and Pathways at Play

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Background: **Epithelial-mesenchymal transition (EMT) is a biological process where epithelial cells acquire mesenchymal traits. Epithelial cells are characterized by tight cell-cell adhesions and apical-basal polarity, whereas mesenchymal cells are generally elongated in appearance with loose cell-cell interactions, allowing increased cell migration. Many studies have been done on EMT pathways in oral cavity carcinoma, but there are few studies about the possible clinical implications.**

Aims & Objectives: **This systematic review was carried out to find the clinical implications of EMT in HNSCC and bring together the molecular, genetic, and epigenetic pathways found to be acting on the cadherin switch.**

Materials & Methods: **An extensive search for relevant papers was made on PubMed, Medline, and Google Search. Only good-quality studies pertaining to epithelial–mesenchymal transition in oral cavity cancers in humans were selected. Furthermore, all selected papers were assessed for their clinical relevance.**

Results: EMT has role in prognostication, diagnostic dilemma, margin assessment, mandibular preservation, making OSCC more prone to systemic therapies and planning risk reduction in strategies. **The cadherin switch is regulated by transcription factors like Snail, SLUG, ZEB1, and ZEB2. It is also acted on by epigenetic modifiers. These transcription factors are regulated by multiple pathways like Wnt/β–catenin, PI3K/AKT, MAPK, etc. These pathways are in turn regulated by molecular and external agents like NNK from tobacco smoke, hypoxia, the DDB2 gene, reactive oxygen species (ROS), and melatonin.**

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth-most common malignant tumour in the world. Despite advances in cancer treatment, the mortality rate due to HNSCC is still alarming, and the capacity of invasion/metastasis of this cancer is an important factor associated with its prognosis [1]. Epithelial-mesenchymal transition (EMT) is a biological process where epithelial cells acquire mesenchymal traits. Epithelial cells are characterized by tight cell-cell adhesions and apical-basal polarity, whereas mesenchymal cells are generally elongated in appearance with loose cell-cell interactions, allowing increased cell migration [2].

The process of EMT is divided into three distinct types, i.e., EMT Type 1, 2, and 3. EMT-1 occurs during normal embryogenesis; EMT-2 during wound healing and tissue regeneration; and EMT-3 is associated with cancer progression [3]. At the molecular level, three important mechanisms contribute to the changes in type 3 EMT: a) Downregulation of E-cadherin, b) Changes in the expression of microRNA (miRNA), c) Reorganization of actin and formation of invadopodia [3]. Many studies have been done on EMT pathways in oral cavity carcinoma, but there are few studies about the possible clinical implications.

The aim of our systematic review is to highlight the molecular pathways of EMT and their clinical implications in oral cavity carcinoma.

Materials and Methods

PRISMA guidelines [4] were followed for this systematic review. An extensive search was was done on PubMed, Medline, and Google Search using the search terms “Epithelial mesenchymal transition AND head and neck cancer,” “Epithelial mesenchymal transition,” “head and neck cancer,” and ”humans.” The search time was extended up to the 27th of August 2023. Studies meeting the following criteria were included:

1. Studies on oral cavity cancers in human subjects
2. Full-text articles in English
3. Studies relating to the clinical implications of EMT

The following types of articles were excluded:

1. Systematic reviews and meta-analyses

2. Articles on animal experiments

All articles were screened for relevance using the title of the paper and the terms “epithelial-mesenchymal transition. Thereafter, two authors reviewed the abstracts to select publications on EMT and human subjects only. Finally, the text of the remaining papers was accessed to ensure that they discussed the clinical implications of EMT. A quality assessment of the selected articles was done using the Newcastle-Ottawa tool [5], and articles of good quality were included. This process is shown in the PRISMA flowchart in Figure 1.

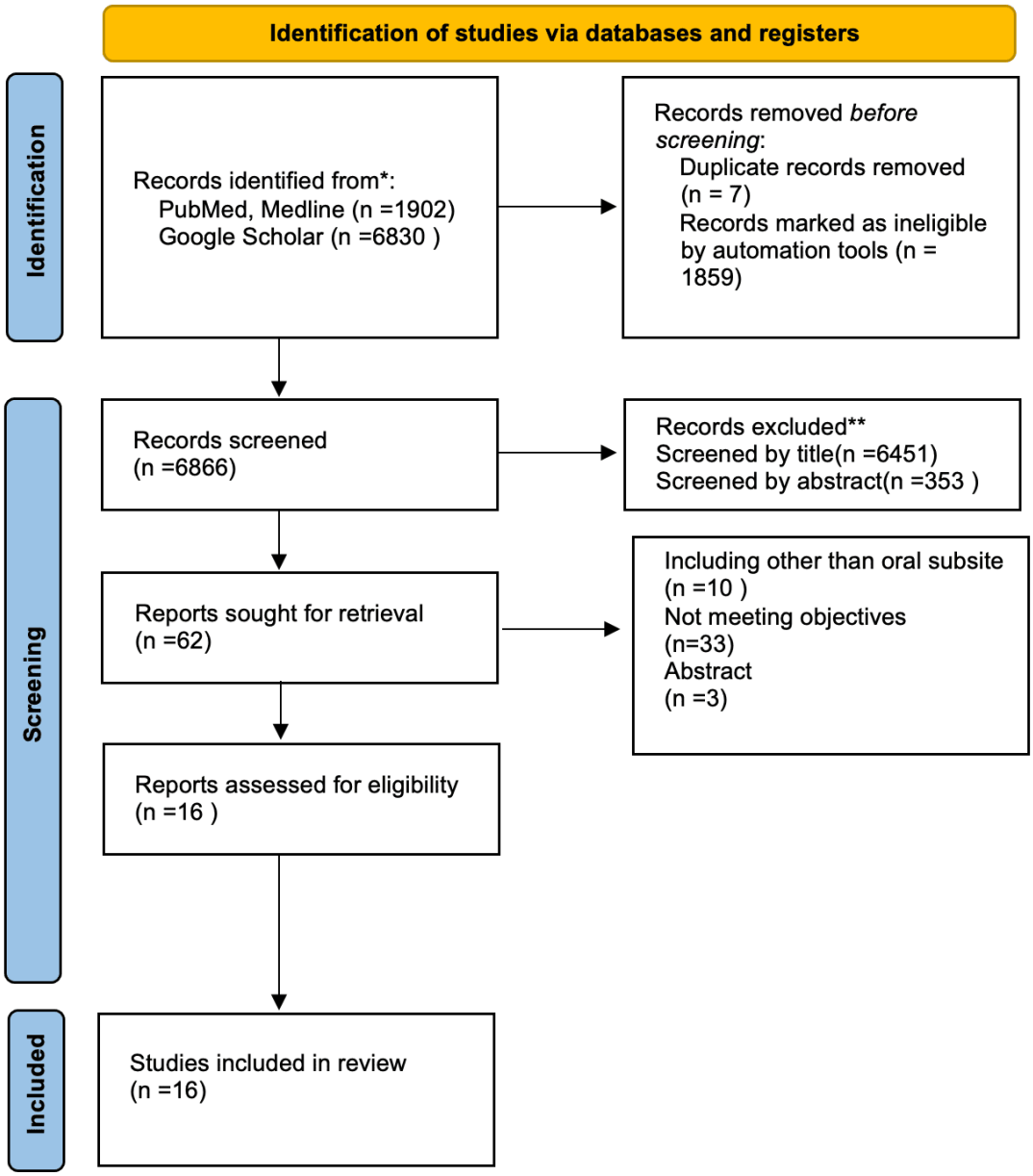

Figure 1. PRISMA FLOW CHART OF ARTICLE SELECTION PROCESS

Results

1902 articles were found via PubMed and Medline, and 6830 via Google search, giving a total of 8732 articles. Seven were removed because they were duplicates, 6451 were screened by title, and 353 by abstract. Finally, full-text retrieval was done on 62 articles. Ten were excluded because they were about sites other than the oral cavity, 33 did not match the objectives of the review, and 3 were only abstracts.

Ultimately, 16 articles of good quality, matching the aims and objectives of the review, were included. The details of these studies are listed in Table 1.

Sr.no.	Study(year)	Pathway	Subject	Number	Quality
1	Ali, Mehwish Feroz (2023)	ZAG-2α	Human	120	Good
2	Bommi, Prashant V (2018)	DDB2	Human	28	Good
3	Gudi, Radhika R (2021)	CPAP	Human cancer cell line	-	Good
4	Huynh, Nam Cong-Nhat(2022)	CAFs	Human cancer cell line	-	Good
5	Kim, Sun(2019)	RON	Human	89	Good
6	Liu, Huijuan(2022)	TAB2	Human	57	Good
7	Liu, Rui(2018)	Melatonin & ROS	Human cancer cell line	-	Good
8	Liu, Xue(2019)	ADAR1	Human	61	Good
9	Nieh, S. (2015)	NNK & Tobacco	Human cancer cell line	-	Good
10	Park, Junhee(2019)	CCL28-induced RARβ	Human	117	Good
11	Rai, K. H. (2019)	N – Cadherin expression	Human	130	Good
12	Wu, Qiuyu(2022)	Chordin like-1	Human	30	Good
13	Yaginuma, Tatsuki(2020)	P130Cas	Human	5	Good
14	Zhang, Hong(2016)	PDGF-D/ PDGFRβ	Human cancer cell line	-	Good
15	Zhang, Y. (2020)	PGK1 & Hypoxia	Human	92	Good
16	Zhou, Xuan(2015)	MALAT1	Human	54	Good

Table 1. Table showing details of studies included, the molecular pathway examined and result of Quality assessment of each study

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Discussion

The epithelial-to-mesenchymal transition (EMT) is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties. It typically occurs during tumour progression and correlates with an increase in local invasiveness and metastatic potential of the tumour.

The studies discussed the pathways that lead to metastasis and bone invasion. Some pathways are more important in margin assessment, while others are more important in the diagnosis of less differentiated early tumours. Hence, further discussion has been divided into the important roles played by them.

Prognosis

EMT has been an indicator of prognosis in carcinomas of many organ systems. Interactions with cancer-associated fibroblasts (CAFs) result in modification of the tumour microenvironment, leading to the formation of partial epithelial – mesenchymal transition (p-EMT) of tumour cells. Partial EMT cells exhibit both epithelial and mesenchymal signatures and play a vital role in tumour progression and metastasis. Huynh et al.[6] used a single-set RNA sequence dataset for OSCC cell lines, GSE103322 and GSE164690, from the Gene Expression Omnibus database and were able to identify four subtypes of CAFs fibroblasts and extracellular matrix gene restructuring, which they labelled eCAF. These cells also expressed cancer-associated genes, e.g., SFRP2 and proteoglycan LUM. In metastatic conditions, they detected increased signalling between eCAFs and p-EMT and the WNT and NOTCH signalling pathways. Thus, they have described CAF-mediated tumour microenvironment (TME) modelling, which helps promote invasion and metastasis.

According to Liu H et al. [7], Transforming growth factor β1-activated kinase 1 binding protein 2 (TAB2) mediates a variety of biological processes through activated nuclear factor κ-light-chain-enhancer of activated B cell (NF-κB) signalling pathways. TAB2 expression was shown to be inversely associated with prognosis. Higher epidermal growth factor receptor (EGFR) signalling can contribute to tumour metastasis and resistance to therapies in oral squamous cell carcinoma (OSCC). Mechanistically, this occurs via the PI3K/AKT pathway, resulting in cell proliferation and inhibition of apoptosis.

Loss of expression of a microtubule/tubulin binding protein, centrosomal protein 4.1-associated protein (CPAP), leads to an increase in EGFR levels and its signalling, hence amplifying the EMT features and

invasiveness of OSCC cells[8]. Simultaneously, cells that are undergoing EMT express lower levels of EGFR and are less susceptible to EGFR-targeted therapies, leading to chemotherapeutic resistance.

Epithelial cells typically express E-cadherin, whereas N-cadherin is expressed by mesenchymal cells. As the tumour progresses from well-differentiated to poorly-differentiated, there is a progressive increase in N-cadherin expression[9]. The authors suggest that N-cadherin expression in OSCC can be considered an accurate marker of EMT.

As per Kim S et al. [10], knockdown of the receptor tyrosine kinase recepteur d'origine nantais (RON) decreases the expression of SLUG, an epithelial-mesenchymal transition (EMT)-related transcription factor, and the phosphorylation of MAPK signalling proteins in human OSCC cells. This blocks cell invasion and migration. This indicates that RON contributes to EMT via the SLUG and MAPK pathways. It provides a theoretical basis for various RON-targeting agents being studied in the therapy of OSCC.

Liu X et al. [11] showed that upregulation of ADAR1 may promote OSCC progression by facilitating the maturation of oncogenic miRNAs and EMT. Adenosine deaminases acting on RNA (ADARs) are involved in adenosine-to-inosine (A-to-I) editing, tumorigenesis, and prognosis [11]. It is postulated that ADARs, particularly the ADAR-p110 short isoform, are more abundant in OSCC and may be acting by maturation of miRNAs and EMT.

Chemokine (C-C motif) ligand 28 (CCL28) [12] leads to induced E-cadherin expression and reduced nuclear localization of β-catenin in OSCC cells with detectable RUNX3 expression levels. This is the result of CCL28-inhibited invasion and epithelial-mesenchymal transition (EMT) [12]. CCL28 is a chemokine constitutively produced by epithelial cells of various mucosal tissues, but its role in various human cancers is controversial. Under physiologic conditions and during inflammation or infection, it contributes to host mucosal defence. The authors, in their experiment, demonstrate that downregulation of CCL28 in OSCC cells reduces RARβ expression, improving their invasive ability.

Damaged-DNA binding protein 2 (DDB2) senses DNA damage and is important in Global Genomic Repair (GG-NER). DDB2 is encoded by the nucleotide excision repair gene and is required for the recognition and removal of UV-light-induced DNA lesions. DDB2 is a critical repressor of EMT-regulatory factors irrespective of cell type or tumour type [13]. The authors have shown that lower DDB2 expression leads to increased expression of pro-EMT transcription factors SNAIL and ZEB1. They suggest that monitoring DDB2 expression may be of therapeutic benefit in patients with advanced OSCC.

Melatonin decreases the migration and invasion of oral cancer cells by the repression of EMT. It is a known potent antioxidant which scavenges a variety of free radicals directly and stimulates the action of antioxidative enzymes indirectly. This requires activation of the ROS-dependent AKT pathway [14].The key findings of the experiments of Liu et al. are

1. Melatonin suppresses ROS production in oral cancer cells
2. It reduces proliferation and apoptosis escape of OSCC by inactivation of ROS-dependent Akt signalling.
3. It inhibits migration and invasion of OSCC mediated by ROS-reliant Akt signalling
4. It abolishes tube formation by HUVECs induced by ROS-activated Akt and ERK pathways.
5. Melatonin hampers oral cancer tumorigenesis in vivo

Platelet-derived growth factor D (PDGF-D) signalling is crucial during the development and progression of human malignancies through interaction with the receptor of PDGF-D (PDGFR). It has been implicated in the tumorigenesis of breast cancer, colorectal cancer, hepatocellular cancers, head and neck squamous cell cancers, lung cancer, prostate cancer, pancreatic cancer, and renal cell carcinoma. It interacts with its cognate receptor PGDFR and cross-talks with NOTCH-1 and NF-κB pathways to promote EMT and tumour growth. A study by Zhang et al. [15] suggested that the EMT process can also be triggered by the PDGF-D/PDGFRβ axis in TSCC (tongue SCC) and then be involved in tumour cell invasion via activation of the p38/AKT/ERK/EMT pathway.

Chronic exposure to the carcinogen 4-methylnitrosamino-1-3-pyridyl-1-butanone (NNK), found in tobacco smoke, is involved in the progression of head and neck squamous cell carcinoma. This current study stated that long-term NNK exposure plays a role in HNSCC by increasing anti-apoptosis and therapeutic resistance via the Snail-RKIP signalling pathway [16]. The data also suggests that targeting the α7 nicotinic acetylcholine receptor or the Snail pathway may prevent the progression of HNSCC.

Zhou X et al. [17] found that MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) is overexpressed in OSCC tissues compared to normal oral mucosa by real-time PCR. MALAT1 promoted cell migration and invasion via regulating the nuclear translocation of NF-κ B and β-catenin, and subsequently EMT in OSCC. It is one of the many long non-coding RNAs, the abnormal expression of which has been found to contribute to carcinogenesis, metastasis, stem cell differentiation, and resistance to chemotherapy and radiotherapy.

Zinc-2α glycoprotein is a clinically active protein and plays a role in tumour formation and proliferation. It was first identified as a lipid-mobilising factor and later as a tumour marker in cancer-induced cachexia. Ali MF et al. [18] showed that high expression of Zinc-alpha 2 glycoprotein (ZAG) is associated with early-stage OSCC, smaller tumour size, absence of lymph node involvement, and well-differentiated tumours. The authors suggest that ZAG can be investigated as a reliable biomarker to maintain the epithelial phenotype, predict prognosis, and post-treatment outcomes.

According to Wu Q et al. [19], CHRDL1 was significantly downregulated in OSCC tissues. CHRDL1 knockdown enhanced migration, invasion, adhesion, and EMT. Overexpression of CHRDL1 had the opposite effect. Chordin like-1 is the antagonist of BMP (Bone morphogenetic proteins) proteins belonging to the TGFβ family, which play an important role in tumour development, progression, and invasion. CHRLD1 has been proven to block BMP-induced increases in breast cancer migration and invasion. With this study, Wu et al. prove the modulatory role of CHRLD1 in oral cavity cancers. Upregulation of CHRLD1 has been found to reverse the epithelial-mesenchymal transition and inhibit metastasis. All of these receptors, markers, and pathways end up acting on the 'cadherin switch'.

The cadherin switch, i.e., the switch from expression of E-cadherin to N-cadherin, plays a central role in reprogramming the cell from an epithelial to a mesenchymal phenotype [20].

The switch is regulated by several key pathways, e.g., transcriptional pathways like the Snail family, ZEB family, and Twist, growth factor signalling pathways like TGF − β[21], Wnt/β − catenin pathway[22], the NOTCH pathway[23], epigenetic changes, and some microRNAs [24][25]. Hypoxia, too, plays an important role in EMT via HIF − α, a key transcription factor. HIF − α acts by regulating the expression of genes involved in angiogenesis, erythropoiesis, metabolism, and cell survival while cross-talking with other important pathways like PI3K/AKT and MAPK [26].

A diagrammatic representation of these pathways is shown in Figure 2. Table 2 lists the factors discussed, their putative mode of action, and their effect on EMT. The DDB2 gene and CAF (cancer-associated fibroblasts) both act on the TGFβ pathway, but CAFs also act on the Wnt and NOTCH pathways. Receptor Tyrosine Kinase (RON) and CHRLD-1 both act on the MAPK pathway, while RON also increases the expression of SLUG, promoting EMT. The Centrosome Protein 4.1-associated protein (CPAP) acts via the EGFR pathway. Reactive oxygen species (ROS), TAB2, PGK1, and PGDF/PGDFR act via the PI3K/AKT pathway, but the PGDF/PGDFR also acts on the NOTCH pathway, which has significant cross-talk with the PI3K/AKT pathway. Melatonin acts by mopping up ROS and suppressing the PI3K/AKT pathway. MALAT1

has been found to act via the Wnt/β − catenin pathway. NNK from tobacco smoke acts on the Snail-RKIP pathway. All the above changes end up modulating the cadherin switch by regulating Snail, SLUG, ZEB1, and ZEB2, transcriptional factors.

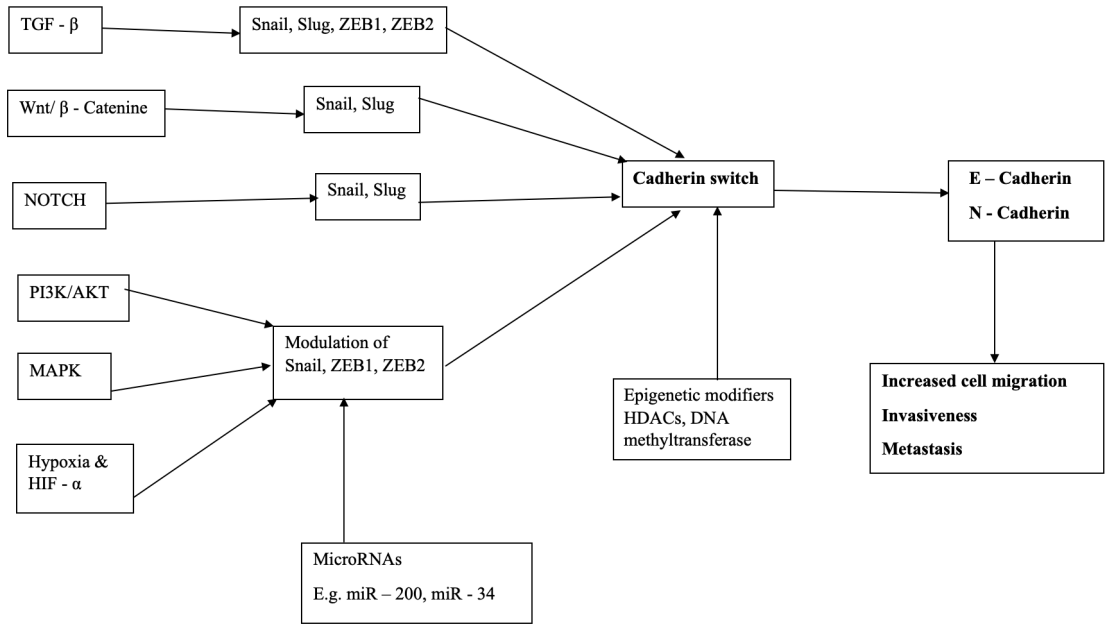

Figure 2. Schematic diagram of the cadherin switch and the pathways acting on it.

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Investigated Factor	Pathway	Result
DDB2 gene	TGFβ	EMT ↘
Centrosomal protein 4.1 – associated Protein(CPAP)	EGFR & phosphoEGFR	EMT ↘
Cancer Associated Fibroblasts (CAF)	Wnt/NOTCH	EMT ↖
Receptor Tyrosine Kinase (RON)	MAPK	EMT ↖
TGFβ activated kinase -1binding protein 2 (TAB2)	PI3K/AKT	EMT ↖
Reactive oxygen species (ROS)	PI3K/AKT	CEMT ↖
Melatonin	ROS & PI3K/AKT	EMT ↘
Adenosine deaminase acting on RNA (ADAR)	MicroRNA	EMT ↖
4-methylnitrosamine-1-3-pyridyl-1-butanone(NNK)	Snail RKIP	EMT ↖
Chordin – like 1 (CHRDL-1)	MAPK	EMT ↘
PGDF & PGDFR	NOTCH & PI3K/AKT	EMT ↖
PGK1	PI3K/AKT	EMT ↖
MALAT1	β - catenin	EMT ↖

Table 2. List of genetic and epigenetic factors discussed in the review, their putative path of action and their effect on EMT

Bone invasion

Bone resection has its functional implications in patients with OSCC. Insights from a study by Park J et al. showed that downregulation of CCL28, CCR10, or RARβ expression was closely related to bone invasion. CCL28 upregulation in OSCC cells can be a new strategy for inhibiting and treating OSCC cell invasion and osteolysis [12]. A study by Yaginuma T et al. concluded that TGF-β1 might enhance the aggressiveness of OSCC, and p130Cas was strongly involved in TGF-β1-induced increase in migration, invasiveness, cell proliferation, and bone invasion. The regulation of p130Cas expression might be a suitable target for the prediction of bone invasion in OSCC patients [27]. These receptors and pathways can be investigated as potential therapeutic targets.

Margin assessment

Ali et al. found that Zinc-α2 Glycoprotein staining occurred only in samples of early squamous cell cancers. Positively stained samples were less differentiated, showing that ZAG expression decreased with increased tumour cell proliferation and differentiation. This may also aid a surgeon in achieving tumour-free surgical margins [18].

Second primary risk assessment

Kim S et al. found that patients with high RON expression tended to have a second primary malignancy more often [10]. This can be used in post-treatment surveillance to screen high-risk patients for second primaries.

Role of hypoxia

A study by Zhang Y et al. revealed that expression of PGK1 was significantly upregulated 12 and 24 hrs following culture under hypoxic conditions. Furthermore, silencing PGK1 expression in hypoxic conditions also significantly inhibited cell migration and invasion [28].

Hypoxia is common in residual tumours after radiotherapy, which is able to thrive in the absence of a blood supply. HIF-α can be investigated as a potential therapeutic target.

Tobacco smoke and NNK

A study by Nieh et al. suggests that long-term NNK exposure increases tumour progression via the Snail-RKIP signalling pathway [16]. The authors suggest that the α-7 nicotinic acetylcholine receptor might be a useful target to prevent progression of squamous cell cancers.

Conclusions

This systematic review investigated the clinical implications of EMT in oral cavity carcinoma. The EMT can be useful in many ways regarding the management of OSCC.

- EMT can be used to prognosticate the OSCC as backed by many studies.
- Markers and pathways of EMT related to early stages of carcinoma can be useful for diagnostic dilemma and margin assessment.

- organ preservation without affecting the oncologic outcomes is a topic of particular importance in every carcinoma. Preservation of the mandible and maxilla can be of utmost importance in terms of functional outcomes of oral cancer management.
- In LA-OSCC targeting the tumor hypoxia-related pathways can lead to better response towards traditional neo-adjuvant therapies, as well as, de-escalation as per need.
- Margin assessment post-neo-adjuvant therapy can become easy using specific markers-based investigations and histopathological analysis.
- Predicting and targeting the risk of second primary and recurrence can be a possibility soon, as the research on EMT advances.
- Risk reduction strategies can be planned for smokers as well as management of carcinoma in smokers can be improved diluting the effects of field cancerisation.

Therefore EMT is opening a new world of management of quite a notorious group of cancers of the oral cavity. Further research on EMT can lead to better oncological and functional outcomes in OSCC.

PRISMA 2020 Main Checklist

Topic	No.	Item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Section 1, Page 3, line 63
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Section 1, Page 3, Lines 63 - 64
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Section 1, Page 3, Lines 63 - 64
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Section 2, Page 3, Lines 67- 69
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Section 2, Page 3, Lines 67- 69
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Section 2, Page 3, Lines 67- 69
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Section 2, Page 3 - 4, Lines 70 - 76

Topic	No.	Item	Location where item is reported
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Section 2, page 4, lines 77 - 80
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Section 2, Page 3, Lines 67- 69 & Table 1
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	LiSection 2, Page 3, Lines 67- 69 & Table 1ne XX-ZZ
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Section 2, page 4, line 81
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Literature review only
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item 5)).	Section 3, page 4, lines 84 - 89
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Literature review only

Topic	No.	Item	Location where item is reported
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Line 89, Table 1
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	No meta-analysis
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	No metaanalysis
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	none
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Newcastle - Ottawa tool, line 81
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	none
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Section 3, page 4, lines 84 - 89
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Prisma Flowchart, Page 4, lines 84 - 89
Study characteristics	17	Cite each included study and present its characteristics.	Table 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Table 1

Topic	No.	Item	Location where item is reported
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	none
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	none
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	none
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	none
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	none
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	none
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	none
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Section 4 , Pages 4 - 11, lines 99 - 257
	23b	Discuss any limitations of the evidence included in the review.	Pages 12 - 14, Lines 259 - 306
	23c	Discuss any limitations of the review processes used.	Pages 12 - 14, Lines 259 - 306
	23d	Discuss implications of the results for practice, policy, and future research.	Pages 12 - 14, Lines 259 - 306

Topic	No.	Item	Location where item is reported
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	not registered
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Protocol not prepared
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	The molecular pathways acting on cadherin switch was added after the articles had been accessed
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	none
Competing interests	26	Declare any competing interests of review authors.	none
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	with corresponding author

PRIMSA Abstract Checklist

Topic	No.	Item	Reported?
TITLE			
Title	1	Identify the report as a systematic review.	No
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	No
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Yes
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	No
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Yes
Synthesis of results	6	Specify the methods used to present and synthesize results.	Yes
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Yes
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			

Topic	No.	Item	Reported?
Funding	11	Specify the primary source of funding for the review.	Yes
Registration	12	Provide the register name and registration number.	Yes

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al.. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. MetaArXiv. 2020, September 14. DOI: 10.31222/osf.io/v7gm2. For more information, visit: www.prisma-statement.org

Statements and Declarations

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Conflict of Interests

The authors declare that they have no conflict of interest.

References

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